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An exploratory study of benthic diatom communities in stormwater ponds of different land uses and varying biocide contamination

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Abstract Stormwater retention ponds receive a variety of urban and highway pollutants that may have adverse effects on water-dwelling organisms. In this exploratory study, the benthic diatom community composition at genus level of nine such ponds servicing highway, residential, industrial, and mixed industrial/residential catchments was examined. Thirteen biocides were measured in the pond water as one of the possible explanatory factors for diatom taxonomic variability. The uppermost 1 cm of sediment was sampled, and a total of 50 diatom genera were identified. Moderate to high similarities were found

among the diatom communities of the ponds. Two genera, namely *Navicula* and *Nitzschia*, were the most abundant and accounted for 19–47% of the relative abundance in the ponds. Estimated relative abundances of diatom genera and measured biocide concentrations in the ponds were grouped according to land use. Highway ponds were found to be significantly different from ponds servicing residential and industrial catchments, while no significant differences were found between residential and industrial ponds. The presence of biocides alone could not explain diatom taxonomic variability, although some evidence was found that communities differed depending on the catchment type of the ponds. The results of this exploratory study are an important contribution to future works investigating stormwater diatom communities, where combined effects of biocides and other stormwater contaminants and community stressors, e.g., metals, PAHs, road salt, should be explicitly looked at.

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Introduction

Increasing urbanization and human activities create pollution pressure on the natural environment. Thus, a number of regulations, directives, and guidelines have

been established to examine and maintain a good ecological status of various terrestrial and aquatic ecosystems (WFD 2000; DEQS 2008; GCERA 2008). Surface water bodies are habitats for a diversity of fauna and flora. Therefore, they receive particular attention with respect to contamination. Investigations of rivers, streams, and lakes over the years have resulted in a number of data sets, providing an insight into water chemistry parameters and their relationship with the living organism community structure (Benetti et al. 2012; Martín and Fernandez 2012; Pereira et al. 2012).

In urban areas, artificial reservoirs, basins, and wetlands are commonly constructed to serve as stormwater detention and treatment facilities, or as a recreational element of an urban environment. One common type is the stormwater retention pond constructed to detain stormwater and retain its pollutants (Hvitved-Jacobsen et al. 2010). Surface runoff contains a substantial amount of particulate and dissolved pollutants, originating from different sources such as vehicles, pavements, installations, and buildings (Bannerman et al. 1993; McLeod et al. 2006). One such pollutant group that has received increasing attention—and which has been found in significant concentrations in urban stormwater runoff—is biocides from building materials (Bollmann et al. 2014).

Biocides are added to a range of building materials to prevent algal and fungal growth, which otherwise would cause discoloration or even degradation of surfaces (Paulus 2004). During rain events, these biocides are released from the materials and transported in the stormwater runoff to ponds or recipients (Burkhardt et al. 2007). For instance, Bollmann et al. (2014) document biocide concentrations in stormwater runoff at a high temporal and spatial resolution and found a wide range of these substances. Highest median concentrations were reported for carbendazim and terbutryn, namely 45 and 52 ng L⁻¹, respectively. During peak events, they occurred in concentrations as high as 306 and 1840 ng L⁻¹, respectively. Other biocides occurred in concentrations between 1 and 10 ng L⁻¹, but with occasional peaks of approx. 100 ng L⁻¹. Generally, the concentration of pollutants in the incoming water varies orders of magnitude between events, with the degree of variation depending on the pollutant type (Leisenring et al. 2014).

The degree of contamination in stormwater ponds also depends on the fate of pollutants. Suspended solids and pollutants associated with particles are

partially removed from the water column by sedimentation (Stanley 1996; Karlsson et al. 2010b). Received loads of nutrients can be, for instance, reduced by pond plant and algae uptake (Hurley and Forman 2011; Chang et al. 2012; Gold et al. 2019). Some pollutants, such as dissolved organic matter (McEnroe et al. 2013a), certain herbicides (Bois et al. 2013), and pharmaceuticals (Liu et al. 2019), are also to some extent susceptible to biological and chemical degradation. Numerous studies report significant pollutant removal, for instance of 80% for metals, 70% for phosphorus, and 30% for nitrogen, depending on the period of the year (Starzec et al. 2005). Thus, stormwater ponds are widely recognized as pollutant retention and accumulation basins (Karlsson et al. 2010a; Weinstein et al. 2010).

Being an integral part of the urban environment, stormwater retention ponds in time also turn into habitats for a variety of aquatic flora and fauna (Scher and Thierry 2005; Le Viol et al. 2009; Sun et al. 2019). However, factors like high loads of anthropogenic pollutants, high nutrient loads, short hydraulic residence time, and high water column mixing rates make these habitats quite different from natural lakes (Hvitved-Jacobsen et al. 2010). Only a few studies have addressed how these differences affect ponds in terms of habitats for aquatic flora and fauna. Among these, Stephansen et al. (2016) studied and compared stormwater ponds and small shallow lakes in Denmark with respect to their invertebrate diversity and bioaccumulation of heavy metals. The authors found similar invertebrate populations in both types of water bodies despite a higher heavy metal bioaccumulation in pond invertebrates. In a study by Hassal and Anderson (2015), stormwater pond macroinvertebrate communities were compared to those of natural wetlands. Although the water quality parameters of the two types of water bodies were quite different, the macroinvertebrate biodiversity was found to be similar in the richest ponds and the natural water bodies. Le Viol et al. (2009) studied macroinvertebrate biodiversity in highway ponds and compared it with that of the surrounding natural ponds. The authors reported similar macroinvertebrate communities in all investigated ponds, despite the measured differences in their abiotic conditions. These and other studies indicate that ponds constructed for stormwater management have the potential to positively contribute to local and regional biodiversity and not only be pollutant retention facilities.

The few studies that have been published on this topic mainly cover invertebrates (e.g., Le Viol et al. 2009; Stephansen et al. 2016), vertebrates (e.g., Ackley and Meylan 2010; Brand et al. 2010), and macrophytes (e.g., Istenič et al. 2012). However, aquatic ecosystems are complex, with a range of different biotic and abiotic parameters governing their behavior. Analysis of a plant and animal community composition of a water body can provide valuable knowledge about ecosystem health or indicate changes in environmental conditions (Parmar et al. 2016). For instance, increased nutrient concentrations are well reflected by shifts in phytoplankton or macroinvertebrate population taxonomic composition (Smith et al. 2007; Parmar et al. 2016). Heavy metal contamination can be indicated by certain macrophyte species (Ladislas et al. 2012; Phillips et al. 2015). While invertebrates, vertebrates, and macrophytes of stormwater ponds have been studied in some detail, algae have received rather limited attention, even though they are of a particular importance to the aquatic ecosystems as they form the basis of the aquatic food webs (John et al. 2011). Especially, those benthic algae associated with bottom sediments, such as diatoms, can be exposed to elevated levels of pollutants and have to either adapt to the environment or be eliminated (Gallagher et al. 2011).

Diatoms are well known for their fast response to changing environmental conditions, which is usually well reflected by changes in diatom species composition (Kelly et al. 1998; Martín and Fernandez 2012). They are good indicators of different water quality parameters, such as nutrient levels, acidity, and turbidity and are widely used for the assessment of the ecological status of water bodies, particularly for routine monitoring of streams and rivers (Kelly et al. 1998; Martín and Fernandez 2012). A number of authors investigating wetland and lake benthic diatom communities also report a close correspondence of certain taxa presence and distribution in the sediment to the measured elevated levels of water column nutrients and salinity (Gell et al. 2002; Zalat and Vildary 2005; Della Bella et al. 2007). A variety of diatom taxa found in the upper centimeter of the sediments of the ponds might hence be related to a range of experienced environmental conditions of the near past.

As described above, effects of physicochemical parameters on benthic diatom assemblages in

freshwater bodies are widely studied and well defined in a number of scientific works and reports. With respect to the growing importance of biocide contamination, to our knowledge, there is a lack of studies focusing on benthic diatom community sensitivity to these contaminants in lakes and stormwater retention ponds. On the other hand, a number of laboratory assessments of pesticide toxicity to diatoms on a single species level or to laboratory-grown biofilm diatoms have been reported. For example, Moisset et al. (2015) showed that the biocide diuron at concentrations of 1 and 10 $\mu\text{g L}^{-1}$ impacted the photosynthetic activity and altered the growth pattern of *Eolimna minima*, *Nitzschia palea*, and *Planorhynchium lanceolatum*. Proia et al. (2011) found increased biofilm diatom mortality and inhibited photosynthesis by diuron at concentrations up to 15 $\mu\text{g L}^{-1}$. Debenest et al. (2009) studied periphyton exposure to herbicides isoproturon and s-metolachlor in controlled microcosm experiments and reported biomass growth inhibition at herbicide concentrations of 5 and 30 $\mu\text{g L}^{-1}$. Regarding taxonomic identification, the authors also found that several diatom species, such as *Melosira varians*, *Nitzschia dissipata*, and *Cocconeis placentula*, were tolerant to the tested herbicide concentrations.

In the present exploratory study, we investigate the taxonomic composition of benthic diatom communities at genus level in sediments from stormwater retention ponds from catchments of different land use. We chose to focus on one of the emerging contaminants, namely, biocides from building materials, some of which are algaecides and hence very likely could influence the survival or otherwise cause changes in diatom communities. We aimed to investigate whether the presence of these contaminants could explain the examined diatom taxonomic variability. With this work, we intended to establish a solid basis for future studies to investigate stormwater diatom communities on a larger scale.

Materials and methods

Sampling sites, sediment extraction, and biocide measurements

For benthic diatom analysis, nine stormwater retention ponds were sampled in August 2014 in Northern and Central Jutland, Denmark (Fig. 1). Samples were

collected when heavy rain disturbance was not present and the water column was back to non-turbulent conditions. In order to cover a range of environmental conditions, ponds were selected in catchments encompassing four different types of land use—highway, residential, industrial, and mixed residential/industrial. Highway ponds are located further away from the built environment and have lower impervious cover in their catchment areas. The separation between residential and industrial areas is based on that the former mainly comprise single-family houses, while the latter contain impervious surfaces of more variable use and buildings of different economic activities. We speculated that highway ponds receive lower concentrations and variety of biocides compared to the industrial and residential ones as there are no obvious biocide-source in these catchments. Pond characteristics are given in Table 1, and the geographical distances among the ponds are provided in the supplementary material Table S1. Sediments were sampled from five

locations distributed along the shore line of each pond, at a water depth of approx. 70 cm. This was done in order to evaluate diatom taxa presence and distribution throughout the pond and whether there were large differences among the identified communities depending on the sampled location in the pond. The stormwater pond water column is generally considered to be rather well mixed, although its homogeneity has been questioned in some works (McEnroe et al. 2013b). Sampling sites are shown in Fig. 1a, and pond sampling locations are exemplified for pond R2 in Fig. 1b.

Sediment samples were collected using a corer consisting of a 5 cm inner diameter plastic cylinder attached to a coring stick. The uppermost 1 cm of sediment was taken from each sample, preserved in ethanol (99.5% VWR Chemicals, 20% final concentration) and stored in cool and dark for further laboratory treatment (Taylor et al. 2007). The final analysis was done within a year of the sampling.

(a) Sampling site locations in Northern and central Jutland, Denmark

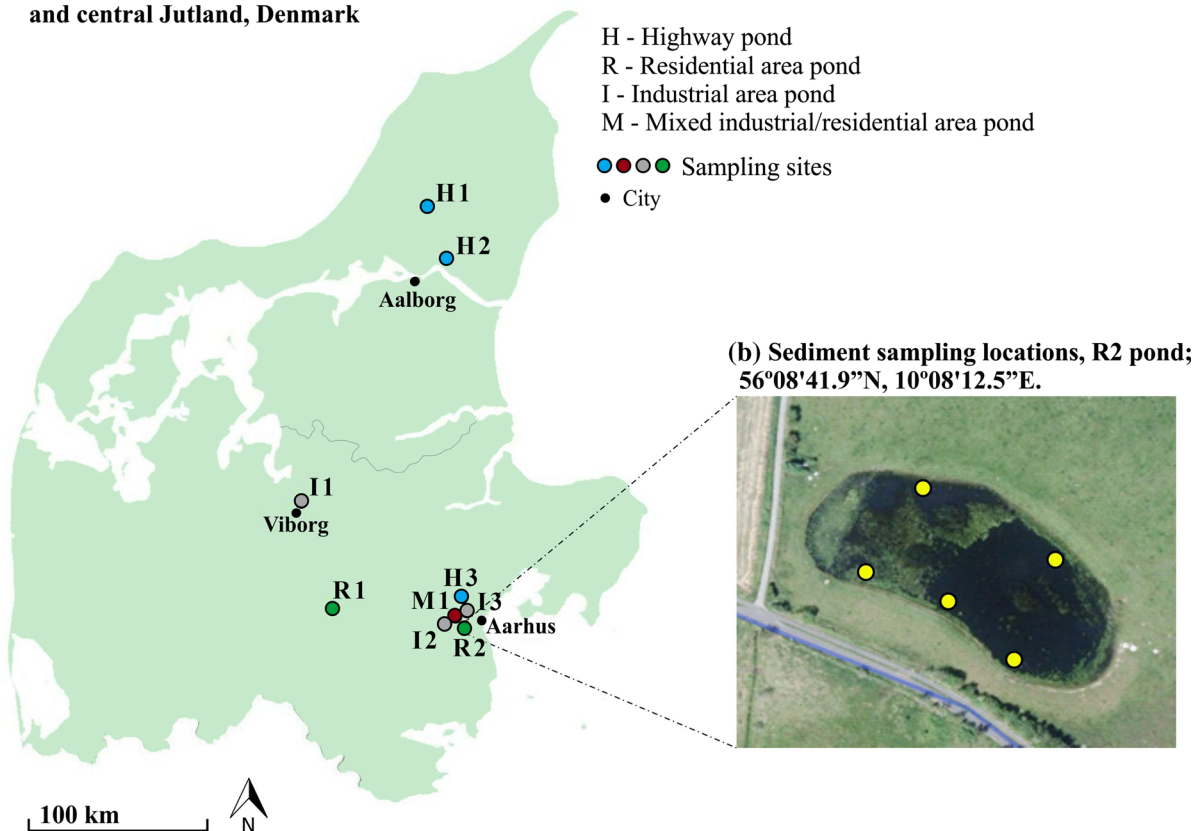


Fig. 1 **a** Sampling sites in Northern Jutland and Central Jutland; **b** sediment sampling locations in one of the ponds (R2)

Table 1 Characteristics of the studied stormwater retention ponds

Pond ID	Coordinates	Catchment type	Pond surface area (m ²)	Pond volume (m ³)	Mean water depth (m)	Catchment area (ha)	Impervious surface area (ha)	Mean water residence time (days)
H1	57° 14' 37.4" N 9° 58' 8.1" E	Highway	4200	2100	0.5	5	4	43
H2	57° 6' 40.3" N 10° 3' 15.6" E	Highway	2340	1170	0.5	8	6	14
H3	56° 13' 13.7" N 10° 7' 43.2" E	Highway	5540	2770	0.5	7	5	41
R1	56° 11' 39.3" N 9° 32' 56.9" E	Residential area	3260	2680	0.8	22	7	27
R2	56° 08' 41.9" N 10° 08' 12.5" E	Residential area	6050	6900	1.1	57	19	26
I1	56° 28' 29.1" N 9° 24' 43.0" E	Industrial area	6500	7800	1.2	166	70	8
I2	56° 9' 32.1" N 10° 3' 30.4" E	Industrial area	6360	7630	1.2	81	21	26
I3	56° 10' 49.5" N 10° 8' 3.1" E	Industrial area	7460	8950	1.2	43	11	58
M1	56° 10' 18.1" N 10° 5' 44.8" E	Mixed residential/ industrial area	11,880	14,330	1.2	92	43	24

When available, parameters were obtained from sewer network engineering diagrams. Alternatively, aerial maps were used together with observations in the field. The mean water residence time was estimated, taking into account an average annual runoff of 14.1 m³ (day impervious ha)^{−1} in Denmark. The average annual runoff was estimated as the average measured precipitation multiplied by a hydrological reduction factor of 0.60

Sediment accumulation rates in stormwater retention ponds are much higher than in natural lakes and depend on different pond and catchment characteristics, such as its size, percentage of impervious surface. Analysis of the uppermost 1 cm of sediment was chosen as an approximation of the more recent history of the stormwater pond diatom population (Schroer et al. 2018).

Water quality parameters, such as pH, dissolved oxygen, conductivity, temperature, nitrite and nitrate nitrogen, total phosphorus, and chlorophyll-*a*, were determined once based on grab sampling of the pond water, simultaneously with the sediment sampling. However, one-time measurements do not represent long-term water column conditions, as ponds are subject to frequent water exchange related with precipitation events and new loads of runoff pollutants and were hence only used for chemical characterization of the ponds and not further discussed nor correlated with the diatom data. All water quality

measurements are provided in the supplementary material.

Water samples for biocide concentration measurements were collected three times as grab samples with 3–4 weeks intervals starting from mid-August. A total of 13 biocides were analyzed: terbutryn (TB), diuron (DR), irgarol (IRG), carbendazim (CD), tebuconazole (TBU), isoproturon (IP), propiconazole (PPZ), methylisothiazolinone (MI), benzylisothiazolinone (BIT), noctylisothiazolinone (OIT), mecoprop (MCP), iodo-propynyl butylcarbamate (IPBC), dichloro-*n*-octylisothiazolinone (DCOIT). Measurements were carried out by means of HPLC–MS/MS, as described in Bollmann et al. (2014).

Diatom slide preparation and analysis

Each sediment sample was well mixed, and a subsample of 3 mL was extracted for further treatment according to the hot 30% H₂O₂ method (Kelly 2000;

DARES 2004; Taylor et al. 2007). After treatment of at least 5 h, any H_2O_2 remaining was washed from the sample by centrifugation and resuspension of the pellet in demineralized water (Kelly 2000; Taylor et al. 2007). A 1.5 mL treated and washed subsample was distributed over a cover slip, previously soaked in nitric acid in order to avoid diatom valve clumping. Then the cover slip was dried and fixed with a mounting agent Naphrax (*Brunel Microscopes Ltd*) on a glass slide. Diatoms were identified to genus level using identification keys, either as books or online data bases (Kelly 2000; Spaulding et al. 2010). At least 300 valves per slide were counted as random fields of view using an upright microscope (Meiji Techno MT6520) with a 1000 \times magnification objective and oil immersion (DARES 2004).

Statistical analysis

Measured biocide concentrations in the ponds were grouped according to the catchment type of the pond and analyzed using one-way ANOVA in SigmaPlot 12.3 (Snedecor and Cochran 1989; Systat Software 2016). The mixed industrial and residential land use was excluded from the ANOVA test as there was only one pond with this land use (Table 1). Where significant differences were found, pairwise post hoc comparisons between the groups were performed using either Tukey's or Dunn's methods, depending on whether the data were normal or not-normal distributed (Conover 1980; Snedecor and Cochran 1989). Pond diatom communities were compared among the three catchment types and tested for significant differences by an analysis of similarities (ANOSIM) test in Primer (Primer E-Ldt 2016). Similarities in species composition among the five sampling locations of each pond were analyzed using Bray–Curtis similarity indices (0 indicates no similarity and 1 indicates perfect similarity) in Past v 3.15 (Hammer et al. 2001). Possible grouping of the nine ponds was tested by Classical Bray–Curtis Cluster analysis and Detrended Correspondence Analysis (DCA) in Past v. 3.15 as well (Hammer et al. 2001; Legendre and Legendre 2012). Correlations among different pond parameters: pond surface area, mean depth, mean water residence time, as well as measured biocide concentrations, and taxonomic diatom data, were analyzed by Spearman's rank correlations (Conover 1980).

Results

Biocide concentrations in the ponds

All measured water column biocide concentrations in the nine ponds are shown in Fig. 2. The numbers are also provided in the supplementary data Table S3.

Four herbicides—mecoprop (MCPP), isoproturon (IP), diuron (DR), and terbutryn (TB), four fungicides—tebuconazole (TBU), propiconazole (PPZ), carbendazim (CD), and n-octylisothiazolinone (OIT), and one broad spectrum antifouling agent—dichloro-n-octylisothiazolinone (DCOIT), were detected. In all ponds, methylisothiazolinone (MI), benzylisothiazolinone (BIT), iodo-propynyl butylcarbamate (IPBC), and irgarol (IRG) were below the detection limit. Concentrations varied from “not detected” to 113 ng L^{-1} depending on sampling time, compound, and pond.

Two biocides—the fungicide TBU and the broad spectrum biocidal agent DCOIT—were detected in all ponds throughout the sampling period. TBU concentrations ranged from 2 to 56 ng L^{-1} , while DCOIT ranged from 3 to 40 ng L^{-1} . Several of the compounds detected at the higher concentrations were herbicides: TB in ponds R1 (28–59 ng L^{-1}) and M1 (36–38 ng L^{-1}), MCPP in M1 (37–57 ng L^{-1}), DR in M1 (58–113 ng L^{-1}), and once in H1 (53 ng L^{-1}), or

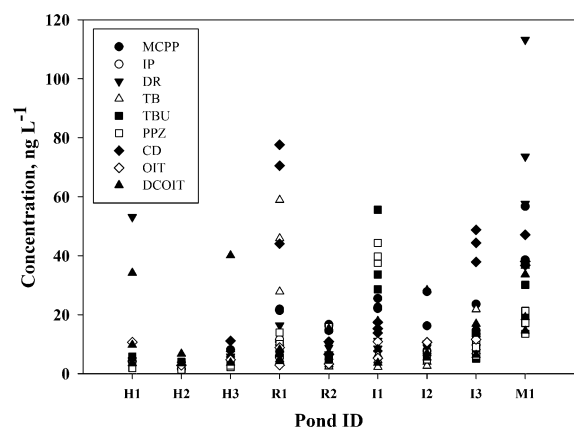


Fig. 2 Biocide concentrations (ng L^{-1}) of the 9 investigated ponds, measured with 3–4 weeks intervals starting from mid-August, 2014. The figure shows all measured data, i.e., 3 values for each substance and pond. Substances below detection limits are not shown. MCPP stands for mecoprop, IP— isoproturon, DR—diuron, TB—terbutryn, TBU—tebuconazole, PPZ—propiconazole, CD—carbendazim, OIT—n-octylisothiazolinone, DCOIT—dichloro-n-octylisothiazolinone

fungicides: PPZ in pond I1 (38–44 ng L⁻¹), CD in R1 (44–78 ng L⁻¹), I3 (44–49 ng L⁻¹), and M1 (37–47 ng L⁻¹). The herbicide IP was detected only in pond R1 and at concentrations of 5 to 12 ng L⁻¹. The concentrations of the antifungal biocide OIT, when detected, ranged from 3 to 12 ng L⁻¹.

Very low or no concentrations of biocides were detected in highway ponds, except in one of the samples from pond H1 where there were 53 ng L⁻¹ of DR and 34 ng L⁻¹ of DCOIT, and one of the sample from H3 with 40 ng L⁻¹ of DCOIT. The highest concentrations of most of the biocides were measured in pond M1, which is located in a mixed industrial and residential area.

Using one-way ANOVA analyses on biocide concentrations, no significant differences were detected between residential and industrial ponds ($p > 0.05$). On the other hand, highway ponds were significantly different both from residential and industrial ponds ($p < 0.05$), except for OIT and DCOIT, where no significant differences were detected among any of the 3 groups. Overall, measured biocide concentrations clearly separated highway ponds from residential and industrial ponds.

Diatom diversity and abundance in the ponds

The five sampled locations were analyzed separately for each pond and subsequently pooled for further analysis. A total of 50 diatom genera were identified in the nine ponds. An overview of the genera, sorted according to the frequency that they were found in each of the water bodies, is given in Table 2. The genera found in 3–5 locations of a pond were considered as common and are marked in grey, while the genera found in 0–2 locations of a pond were considered as absent or rare and are marked in white. The total number of genera identified in each pond is given as a range, e.g., 24–37. The first number indicates how many genera were found in all five sampling locations, while the last number tells how many genera were found in at least one of the locations in a pond.

Similar numbers of genera were found in all nine ponds, ranging from 25 identified at one or more of the five sampled locations of I1 to 40 identified in H2. Only pond I1 could be distinguished from the other ponds to some degree, as it had the lowest number of genera: 15–25. With respect to abundant and dominant

genera (found in 4 or 5 locations of a pond), similar numbers were observed in all ponds: 23–27 in highway ponds, 23–24 in residential ponds, 15–23 in industrial ponds and 28 in the mixed industrial and residential pond. Eight genera, namely, *Fragilaria*, *Gomphonema*, *Navicula*, *Nitzschia*, *Planothidium*, *Sellaphora*, *Surirella*, and *Tabularia*, were frequently observed (3–5 sampled locations) in all nine ponds. The rest of the genera occurrence differed depending on a pond (Table 2), which is highly likely a consequence of habitat diversity, physical and chemical pond parameters, etc.

As the frequency at which a genus was observed within a pond only partly reflects its distribution, the relative abundance of each identified genus was estimated as well. Based on a study of Thayer et al. (1983), all genera were categorized as: absent/very rare (0–1%), rare (1–5%), few (5–10%), common (10–20%), and abundant (> 20%). In order to analyze the genera abundance within a pond, the data were plotted for the 5 sampled locations of each pond (supplementary material, Fig. S2). Only the common and abundant genera were selected in order to have a clearer overview of genera distribution among the locations.

In total 15 genera were found as common (10–20%) or abundant (> 20%) at least in one location of a pond. The list of those genera and their environmental preferences is described in supplementary material (Table S4). These genera accounted for 45–80% of the relative abundance in the ponds. Two genera, namely *Navicula* and *Nitzschia*, were found in high abundances in all locations of the nine ponds. Together they constituted 19–47% of the relative abundance of benthic diatoms in the ponds.

Analyzing common and abundant genera (at least in one location of a pond), a few differences emerged among the ponds (supplementary material, Fig. S2). For instance, several genera were found only in a certain pond: *Diatoma* in H2 (5–12%), *Gyrosigma* in H3 (4–12%), *Asterionella* in R1 (7–11%), *Neidium*, *Pinnularia*, and *Synedra* in I1 (1–11%, 6–15% and 6–15%, respectively), *Planothidium* and *Surirella* in I2 (5–11% and 4–14%, respectively). The five sampling locations of each pond were rather similar in terms of diatom composition, with an exception of *Fragilaria* which was noted as a dominant genus only at one of the five sampled locations of pond H1 (28.9%

Table 2 Investigated stormwater retention ponds and diatom genera identified in the 5 sampled locations of each pond

Genus	Pond ID								
	Highway			Residential		Industrial			Mix
	H1	H2	H3	R1	R2	I1	I2	I3	M1
<i>Gomphonema</i>	5	5	5	5	5	5	5	5	5
<i>Navicula</i>	5	5	5	5	5	5	5	5	5
<i>Nitzschia</i>	5	5	5	5	5	5	5	5	5
<i>Planothidium</i>	5	5	5	5	5	5	5	5	5
<i>Fragilaria</i>	5	5	5	5	5	5	5	4	5
<i>Sellaphora</i>	4	5	5	5	5	5	4	5	5
<i>Surirella</i>	5	5	5	5	5	5	5	3	5
<i>Tabularia</i>	5	5	5	5	5	3	5	5	5
<i>Synedra</i>	5	5	2	5	5	5	5	5	5
<i>Encyonema</i>	5	5	5	4	5	1	5	5	5
<i>Gyrosigma</i>	5	5	5	5	5	0	5	5	5
<i>Hippodonta</i>	5	5	4	5	5	0	5	5	5
<i>Craticula</i>	4	4	3	4	5	3	5	5	5
<i>Cocconeis</i>	5	5	5	1	5	1	5	5	5
<i>Cymatopleura</i>	5	5	5	3	5	1	4	4	5
<i>Luticola</i>	3	2	5	5	4	5	3	5	5
<i>Pinnularia</i>	5	5	2	5	5	5	0	5	4
<i>Amphora</i>	5	5	5	0	5	0	5	5	5
<i>Rhoicosphenia</i>	5	5	5	0	5	0	5	4	5
<i>Tryblionella</i>	3	5	5	0	5	0	5	5	5
<i>Cyclotella</i>	5	5	0	5	1	0	5	5	5
<i>Diatoma</i>	5	5	3	1	1	0	4	5	5
<i>Eunotia</i>	3	5	3	5	1	0	4	5	1
<i>Neidium</i>	0	4	2	5	4	5	1	2	4
<i>Stauroneis</i>	0	2	0	5	5	5	2	5	3
<i>Achnanthes</i>	3	3	2	5	1	5	0	3	4
<i>Lemnicola</i>	5	0	4	0	2	5	5	2	2
<i>Epithemia</i>	5	3	5	0	2	1	2	0	3
<i>Fallacia</i>	3	0	5	0	4	0	3	0	4
<i>Hantzschia</i>	2	1	4	4	1	2	1	2	1
<i>Cymbella</i>	5	3	0	0	0	0	2	3	3
<i>Staurosira</i>	3	3	0	4	0	0	1	1	4
<i>Caloneis</i>	4	4	4	1	0	0	1	0	1
<i>Melosira</i>	5	5	0	0	0	0	5	0	0
<i>Asterionella</i>	0	2	0	5	0	5	0	0	0
<i>Placoneis</i>	0	0	0	0	5	0	0	1	5
<i>Meridion</i>	0	2	0	2	1	0	1	0	4
<i>Frustulia</i>	2	0	1	0	2	3	1	0	0
<i>Rhopalodia</i>	0	0	5	0	2	0	1	1	0
Unidentified	0	2	0	1	2	0	2	1	1
<i>Diploneis</i>	0	1	0	1	0	2	0	2	2
<i>Fragilariaforma</i>	3	1	0	0	1	0	2	0	0
<i>Ctenophora</i>	1	5	0	0	0	0	0	0	0
<i>Anomoeoneis</i>	5	0	0	0	0	0	0	0	0
<i>Cyclostephanos</i>	5	0	0	0	0	0	0	0	0
<i>Stauroforma</i>	0	0	0	0	0	1	1	1	0
<i>Tabellaria</i>	0	0	0	3	0	0	0	0	0
<i>Achnantheidium</i> (<i>& Rossithidium</i>)	0	0	0	0	0	0	0	0	2
<i>Cosmioneis</i>	0	1	0	0	0	0	0	0	0
<i>Staurosirella</i>	0	1	0	0	0	0	0	0	0
Total number of identified genera	24-37	23-40	19-31	19-31	21-36	15-25	19-38	20-35	22-38

Numbers from one to five indicate in how many locations the genus was detected. The genera are sorted according to the observed frequency: 0–2 locations (in white) and 3–5 locations (in grey). Total numbers are given as a range, where the first number points out the number of genera found in all 5 locations of the pond and the second number points out the number of genera found in at least 1 location of the pond

versus 1.7–4.4% in the other locations) (see discussion below).

Bray–Curtis similarity test performed for the different locations of a pond has shown that all locations of a pond were highly similar, with Bray–Curtis values ranging from 0.57 to 0.92. The lowest similarity was found for the one location in pond H1, which was mainly due to the somewhat higher abundance of *Fragilaria*.

Because of the high similarity of the sampling locations, the five locations of each pond were grouped, and the mean relative abundance of each genus was calculated for each pond. The genera were categorized according to their abundance, as described above. In order to have a clearer overview of genera dominance, only the genera commonly found (10–20%) or abundant (> 20%) at least in one of the 9 ponds were selected and plotted (Fig. 3). Only two genera, namely, *Navicula* and *Nitzschia*, were found common or abundant in all nine ponds: 12–22% and 11–20%, respectively. Several other genera found in high abundance (> 10%) were *Amphora* in R2 (12%), *Cocconeis* in all ponds (11–26%) except H1, R1 and I1, *Synedra* in I1 (12%), and *Cyclotella* in R1 and I3 (12% and 11%, respectively).

Statistical Bray–Curtis similarity was compared among the 9 ponds. It ranged from 0.44 to 0.87, indicating moderate to high diatom community similarities among the ponds. Most of the lowest

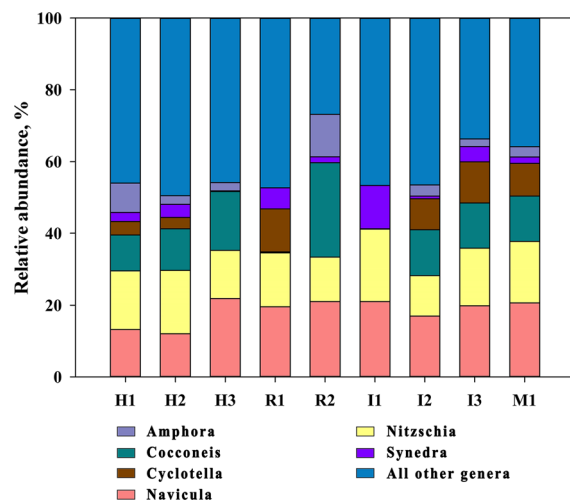


Fig. 3 Common (10–20%) and abundant (> 20%) diatom genera distribution in the nine investigated ponds, as well as the part taken by all other identified genera

similarities were found when comparing ponds R1 and I1 with highway ponds (0.46–0.55). Other moderate dissimilar ponds were R1 and R2 (0.5), R2 and I1 (0.46), and I1 and I2 (0.44). Bray–Curtis similarity indices were also plotted against the geographical distances between the ponds to test whether pond diatom communities had higher similarity for the ponds located closer to one another. No similarity–distance correlation was found (supplementary material, Fig. S1).

A pond comparison was also carried out by an ANOSIM test, where ponds were grouped according to their catchment type (highway, residential, industrial). Dissimilarities in benthic diatom communities among ponds were not large (ANOSIM, global $R = 0.242$). However, some significant differences were found. Highway ponds were found to be different from residential ponds (ANOSIM, $R = 0.359$, $p = 0.002$), as well as industrial ones (ANOSIM, $R = 0.258$, $p = 0.001$), while residential and industrial ponds were not significantly different from each other ($p = 0.087$).

The taxonomic diatom data and estimated genera relative abundances were submitted to a DCA analysis (Fig. 4) to test any possible grouping of ponds and whether it could be related to any of the measured chemical or physical pond parameters. No grouping with respect to catchment characteristics could be observed. The majority of identified genera and the dominant genera were found to be similar for all ponds. Several physical and chemical pond parameters, such as pond surface area, mean water depth, water residence time, and biocide concentration, were correlated with pond DCA axis 1 coordinates. Axis 1 was found to significantly ($p < 0.05$) correlate with

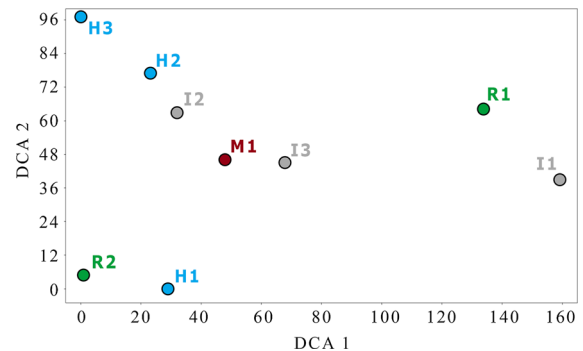


Fig. 4 DCA analysis for the benthic diatom genera relative abundance of the nine investigated stormwater retention ponds

the average concentrations of mecoprop (MCP) ($r_s = 0.73$), terbutryn (TB) ($r_s = 0.80$), and propiconazole (PPZ) ($r_s = 0.77$).

Discussion

Twenty-five (pond I1) to 40 (pond H2) diatom genera were identified in the nine ponds of this study. Numbers were similar in all ponds, except pond I1, which had the lowest number of genera (15–25). This pond is located in an industrial area, and it is probable that it receives a wider range of pollutants compared to residential or highway ponds. Another explanation could be that this pond has the lowest water residence time and the largest catchment area compared to the other ponds. This could result in a more frequent pollutant load and correspondingly higher exposure of diatoms hereto. As a consequence, only the most tolerant diatom communities would remain in the pond (Cattaneo et al. 2011).

Diatom composition at the five sampling locations of each pond was comparatively similar. Only in pond H1, *Fragilaria* stood out as a dominant genus at just one of the five sampled locations. Whether the presence of certain genera only in some locations of a pond could have been caused by location-specific parameters, such as light availability or the presence/absence of macrophytes, or was simply due to random variability, is not known. Some studies also report higher *Fragilaria* species abundance as a consequence of increased agricultural activities and other human caused disturbances (Watchorn et al. 2008; Della Bella and Marcini 2009). Nevertheless, it is rather difficult to apply such findings to the case of the present study, as *Fragilaria* genus was found as dominant only in one of the studied five pond locations.

Additionally, several of the common genera (10–20% at least in one location of a pond), namely, *Diatoma*, *Gyrosigma*, *Asterionella*, *Neidium*, *Pinnularia*, *Synedra*, *Planothidium*, and *Surirella*, were found only in one of the nine ponds. Despite that, the differences among dominant genera did not seem to depend on the land use type of the pond—highway, residential, industrial or mixed residential and industrial. Additionally, regardless of a few differences, the mentioned eight taxa are tolerant to either a range of water pH, salinity, trophy, saprobity or are motile,

which makes them adaptive to various environmental conditions (supplementary material, Table S4).

Although diatom communities in the nine ponds were found to be composed of a variety of genera, only a few of them, specifically, *Amphora*, *Cocconeis*, *Synedra*, and *Cyclotella*, were found at a relative abundance of more than 10% in at least one of the nine ponds. Two genera—*Navicula* and *Nitzschia*—constituted the largest part of the diatom communities in all nine ponds. All six genera are alkaliphilous or tolerate a range of pH values, as well as prefer nutrient rich, eutrophic waters, which is often the case of stormwater ponds. Ponds of the present study fall into categories of eutrophic or hypertrophic waters (Bellingier and Sigee 2010), according to the measured total phosphorus and chlorophyll-*a* concentrations (supplementary material, Table S2). *Navicula* and *Nitzschia* are also motile genera, able to adapt to nutrient rich and turbulent environments (Rimet 2012), and some of *Navicula* species are also listed as pollution tolerant (Kelly 2000). According to Ludikova (2016), higher *Cyclotella* abundance might be related to higher phosphorus concentrations. The same author has investigated 53 urban ponds and lakes of St. Petersburg and found the genera *Achnanthes*, *Cocconeis*, *Cyclotella*, *Gomphonema*, *Lemnicola*, *Navicula*, *Nitzschia*, and *Stephanodiscus* as the most common.

Regarding the measured biocide concentrations in the nine ponds of the present study, they varied from “not detected” to 113 ng L⁻¹ depending on sampling time, compound, and pond (catchment). None of the measurements exceeded the recommended annual average values of the European Water Framework Directive for priority substances in inland waters (WFD 2013). The directive suggests not to exceed concentrations of 64 ng L⁻¹ for TB, 200 ng L⁻¹ for DR, and 300 ng L⁻¹ for IP. The concentrations measured in the present study are several orders of magnitude lower than some of those used in controlled investigations by other authors testing the effect of biocides on diatom communities. For instance, Rimet and Bouchez (2011) carried out a river mesocosm study where diuron and terbutryn were two of the three pesticides used. The total pesticide concentrations ranged from 1.11 to 3.01 µg L⁻¹ for long-term exposure and from 20.25 to 29.50 µg L⁻¹ for acute exposure. These levels caused diatom community shifts toward higher abundances of pollution tolerant

taxa such as *Navicula*, *Nitzschia*, and *Cocconeis*, which exhibit motility or forming of mucous tubules. Despite the lower biocide concentrations in the stormwater retention ponds of the present study, the higher abundances of these diatom genera in the nine ponds of this study might have been influenced by their higher tolerance to contamination. Following Bester et al. (1995), on the other hand, effects of triazine herbicides could be determined down to 100 ng L⁻¹ in a marine ecosystem. Another study performed by Marcel et al. (2013) analyzed a large monitoring data set of river diatoms and correlated their ecological guilds—groups of diatom taxa that prefer certain environmental conditions—with herbicide contamination. The authors found that “high profile” guild diatom abundance, “genera such as *Diatoma*, *Eunotia*, *Fragilaria*, *Gomphonema*, *Gomphonema* and *Ulnaria*,” decreased significantly with an increase in herbicide atrazine concentration. They observed a similar tendency for herbicides diuron and isoproturon as well.

The taxonomic differences of the benthic diatom communities of the present study could not be connected to the measured concentrations of biocides alone, although the statistical analysis indicated that diatom community composition was to some degree influenced by the pond catchment characteristics. One-way ANOVA tests for the measured biocide concentrations showed a clear separation between highway ponds compared to residential and industrial ones, while no significant differences were found between residential and industrial ponds. The same result was obtained by an ANOSIM test, carried out to compare diatom communities of the three types of ponds. The DCA study also provided some evidence that diatom community composition in ponds might be related to biocide concentrations. However, the evidence was not strong enough to discard the possibility that biocide concentration could be merely a proxy of another structuring parameter or set of parameters.

This was probably due to differences in runoff volumes, and runoff patterns of pollutants and nutrients, as well as the potential colonization from adjacent ponds and lakes (Naselli-Flores et al. 2016). The majority of the ponds investigated in this study were separated by only a few kilometers (3–9 km) or tens of kilometers (32–57 km), except for two highway ponds (H1 and H2), which were located further away (81–123 km) from the rest of the sampling sites

(see Table S1). Moreover, sediment type is an important factor influencing diatom species presence and distribution in a water body as well. Linnan et al. (2017) found for instance that fine sediments favor diatom abundance. Similarly, Babeesh et al. (2016) showed a close association of sediment texture and diatom distribution, with species such as *Eunotia pectinales* and *Encyonema latum* negatively correlated with sandy, but positively correlated with muddy, sediments. Although sediment properties were not analyzed in the present study, this aspect could be potentially interesting to consider in the future works as well.

Overall, the results of this study provide evidence that benthic diatom communities were somewhat affected by the catchment land use type of the ponds, especially when comparing highway ponds to residential and industrial ones. The overall diatom taxonomic composition depends not only on the effect of specific pollutants and their concentrations, but also on a combination of different factors such as species forming the initial diatom community, physical and chemical water parameters, morphology of the water body, biological links to other aquatic ecosystems, and etcetera. The rather weak correlation between biocides and ponds, and land use and ponds (as a proxy for pollutant type and concentration) indicates that those other factors play a major role for the overall diatom taxonomic composition of a stormwater retention pond. The results found in this study on the response of benthic diatom communities of stormwater ponds toward contaminants such as biocides indicate that further attention should be devoted to elucidating the combined effects of biocides and other contaminants, such as heavy metals (especially copper), poly aromatic hydrocarbons, road salt.

Conclusions

The benthic diatom community composition of nine stormwater retention ponds located in catchments of different land use and receiving varying levels of biocide contamination was examined. In total, 50 diatom genera were identified with the pollutant tolerant taxa *Navicula* and *Nitzschia* making up a large part of diatom communities in all nine ponds and constituting 19–47% of the relative abundance. According to the measured biocide concentrations, as well as the

estimated diatom relative abundance, highway ponds were found to be significantly different from the residential and industrial ones. However, the taxonomic variations of diatom communities in ponds of different land use type could not be connected to the presence and concentrations of the measured biocides alone.

The results clearly demonstrate that artificial water bodies constructed to treat stormwater prior to discharge to the environment had benthic sediment diatom communities composed of a variety of genera. This suggests that artificial stormwater ponds serve as ecosystems of ecological value. Diatoms are an important part of the ecosystem, forming the base of the food web, and their dynamics should be clearly understood when engineering stormwater ponds not only as treatment facilities but also as assets to enhance regional biodiversity. While the present study is still far from explaining all the observed community patterns, it demonstrates that stormwater pollutants found in our stormwater ponds certainly do not render these water bodies uninhabitable. Nevertheless, biocides might have an impact on the ecosystem, which should be better understood for optimal management of stormwater runoff. Accordingly, further works are needed to examine combined effects of biocides and other stormwater contaminants on diatom communities, such as metals, PAHs, road salt.

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